

A GENETIC STUDY OF THE TRANSPLANTATION OF
TUMORS ARISING IN HYBRID MICE



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INTRODUCTION

Historical Review

A. Heredity of Transplantation: The problem of the transplantation of cancer has held the attention of numerous investigators since 1900. Very few of those who have published in this field are actively engaged in cancer work at the present time. It would appear as if researchers dipped into this field as a side line, hoping that with a few mice secured from various dealers they would arrive at some definite conclusions, and, after a few experiments giving contradictory results, departed for broader fields.

The entire literature on the transplantation of malignant tissue cannot be covered in a thesis of the present nature. I shall consequently limit my discussion to those investigations that bear upon the genetic analysis of the problem.

The first successful transplantation of cancer between members of the same species was accomplished by Novinsky in 1876. He employed a lymphosarcoma of a dog. Out of forty-two inoculations, he secured two growths.

In 1889, Hanau, working with a carcinoma of the rat, succeeded in transferring it to one of two animals inoculated.

Pfeiffer mentioned in a paper published in 1890 that he had been successful in transplanting a melanotic sarcoma to other mice.

Using a spindle-cell periosteal sarcoma of the rat, von Eiselberg, in 1890, secured one susceptible individual out of two inoculated. He described the resulting growth as being more cellular than the original.

Morau, in a series of articles published from 1891 to 1894, described the successful transplantation of an epithelioma of the rat through 17 generations. He was also able to keep alive a cylindrical-cell mouse carcinoma for several generations. The

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transplants developed more rapidly in the progeny of tumor-bearing mice. Morau was the first to point out that heredity may play an important part in the successful transplantation of tumors.

Firket, in 1892, transferred a sarcoma of the rat to other individuals, for four generations. Only six rats were used, five of which were susceptible to the transplant.

Transplanting a spindle-cell sarcoma, Velich, in 1898, was able to carry it through eight generations, but lost the tissue due to the introduction of a foreign strain of rats from another dealer. Previous to inoculation into the new stock, Velich maintained, there had been a progressive decrease in virulence of the sarcoma, so that he did not attribute the loss of the tumor to the introduction of the foreign rats.

Loeb maintained that race played an important part in transplantation. He determined (1901) that a tumor arising in a member of the Japanese waltzing stock of mice would grow, when inoculated, in all individuals of the parent race but not in the common mouse or white variety. Loeb (1902) also showed that when two tissues derived from the same mass were inoculated simultaneously into rats, the hosts grew either both or none.

Jensen (1903) carried a carcinoma of the mouse through nineteen generations. He found that there was a difference in susceptibility between the various races of domestic mice. He also found that there was an increase in susceptibility with the continued inoculation of an albino-mouse tumor into gray mice. At first nine out of ten refused to develop the transplant; later twenty-seven out of eighty-four showed growths.

Haaland (1907), employing a sarcoma arising in a member of the Frankfort stock of mice, found that it would transplant readily into other Frankfort mice and in mice secured from other parts of Germany, but would grow only poorly in Norwegian mice. Haaland later moved part of his stock to Christiania and after several months found that they were resistant to the tumor. He attributed the change in susceptibility to a change in diet. This difference is of little significance, however, since only six mice were employed.

Hertwig and Poll in 1907 denied that heredity played any part in the transplantation of neoplasms.

Cuénot and Mercier (1910), however, found that heredity must be taken into consideration. Out of 83 mice, which were descendants from a susceptible stock, 76 (85.3 per cent) developed

the transplanted tumor, while only 17 (16.5 per cent) of 103 offspring from a non-susceptible stock showed growths. The same investigators later determined (1910) that within one stock there was no difference in susceptibility between individuals of the various color varieties.

In 1908, Gay transplanted the Flexner rat tumor into two strains, one of which gave all susceptible individuals; the other, secured from another dealer, gave successful "takes" in only 50 per cent of those inoculated.

Inoculating Jensen's tumor, which grew best in Danish white mice, Bashford and Murray (1904) found that the English white stock was less susceptible than other races of English tame mice. Bashford, Murray, and Cramer (1905) employed two series of mice derived from the same source. One series received a single transplant and the other series five grafts from the same tissue. The series receiving the single graft gave a smaller number of progressively growing tumors than the one inoculated with the five tissues. From this it was concluded that the influence of the tissue transplanted was much more important than that of the host.

Haaland (1907) experimenting with an artificially mixed transplant of Jensen's carcinoma and a sarcoma from Ehrlich's laboratory, was able to demonstrate that Danish mice were susceptible to the carcinoma component of the graft but refused to grow the sarcoma element. Berlin mice, on the other hand, grew the sarcoma moiety but not the carcinomatous part of the transplant.

Experiments of the above nature throw little light on the modern theory of transplantation, for several reasons. (1) "Market stocks" were employed as a rule. These were obtained from different dealers and resulted in various complexities, including frequently the loss of the transplant. (2) The numbers of animals employed were too small for valid conclusions. In many experiments two to six individuals made up the population used. It will be remembered that Haaland inoculated six mice from a Frankfort stock which had been moved some time previously to Christiania, and maintained that there was a difference in susceptibility as compared with the original Frankfort stock. Conclusions of this nature were entirely too common. (3) Percentages only were given in some investigations, the number of individuals being omitted. Eighty per cent might mean 80 mice out of 100 or 4 out of 5, as was too frequently the case. (4) Many investigators

claimed that they were experimenting with "inbred stocks," as foreign breeds were not introduced. These stocks gave various percentages of susceptible animals in succeeding series of inoculations with the same tumor. Loeb and Fleisher (1916) stated that they were only using "pure stocks," and yet the number of positive animals in one race varied from 12.5 per cent to 86 per cent; in another from 0 per cent to 25 per cent. (5) Since genetically pure stocks properly isolated were not employed, the proper hybrid crosses could not be made and the nature of inheritance, if any existed, demonstrated or even indicated. (6) As the science of genetics was still in its infancy, its principles were not applied. Previous to 1916 no geneticist had been interested in the problem of transplantation.

B. Mendelian Interpretation of Transplantation: The first work of this period was performed by Loeb and has been mentioned previously. Later Loeb, in coöperation with Fleisher (1916), brought forth further evidence in support of the hereditary transmission of susceptibility. Hybrids made by crossing pure stocks showed no variation in susceptibility. The number of individuals used, however, was small, and the variations encountered within the so-called "pure stocks" were too great to be very convincing to any geneticist.

Loeb and Fleisher (1912) advanced the theory that the difference existing between stocks could be explained by the multiple factor hypothesis. To determine this difference, they took the percentage of mice which would grow a tumor as characteristic of that stock. From the difference in the percentages existing between various races they would compute the number of factors involved. This type of analysis is exceptional in the field of genetics. In order to place the data on a convincing genetic basis, the accepted genetic methods for "multiple factor" complexes should have been employed. The nature of the procedure adopted by these investigators will be pointed out later in this paper.

The theory regarding the transplantation of normal tissue has been placed on a similar basis by Loeb (1920). According to the pedigree relationship, he has employed the following terms to represent the various types of transplantation (for both neoplastic and normal tissue); autotransplantation, transfer of tissue from an individual to another part of itself; syngenesiotransplantation, transfer between closely related individuals; homoiotransplantation, between totally unrelated individuals or animals of different

species. To account for the variations arising among hosts, Loeb assumes an "individuality differential," which he believes to be of a chemical nature.

Loeb found differences to exist between members of the same families of rats and guinea-pigs. The results were intermediate between homoio- and autotransplantation. He considered the transplantation from offspring to mother as characteristic of homoiotransplantation, since the progeny would have only one-half the chromosome complex of the mother.

Loeb's theory of "individuality differential" has been criticized by Little (1924). The latter claimed that if this difference is of a chemical nature it must be uniform in amount throughout the individual and communicable to all the cells. It is inherited and, therefore, must be present in the germ-plasm. If inbred stocks were employed, as Loeb maintained, all the individuals should be biologically the same.

Little holds that since members of the same race did not give uniform results, no analysis of the presence of multiple factors can be made. The factors involved in transplantation, whether of tumors or normal tissue, can be determined only from the results secured in the F_2 generation derived from pure stocks giving uniform reactions. Thus it appears that scepticism is encountered in the acceptance of Loeb's conclusions.

Loeb (Loeb and Wright, 1927) has modified his theory on transplantation, at least for normal tissue, within the past few years. The conclusions in general were ". . . the number of strange genes . . . in the individuality differential of the transplanted tissue determines the severity of the reaction of the host against the transplant. The presence of strange genes in the individuality differential of the host; or expressed differently, the absence of certain genes in the transplant or the presence of double genes in the transplant does not call forth a reaction in the host."

In 1909 Tyzzer confirmed Loeb's previous experiment by determining that a carcinoma arising in a Japanese waltzing mouse would grow in all members of the parent stock but not in the common house mouse. Hybrids between the two races were all susceptible. Tyzzer raised the F_2 generation, but of the 54 mice inoculated, all were negative to the transplants. He concluded that susceptibility is inherited but not in accordance with any known mendelian hypothesis. He failed in the genetic analysis of this problem of transplantation, as pointed out by Little and Tyzzer later, by not having large enough numbers.

Further data on this tumor were published by Little and Tyzzer (1916). The previous results for the parent and hybrid stocks were the same. Out of 183 individuals inoculated of the F_2 generation, 3 proved to be susceptible. The back-cross generation to the original susceptible stock ($F_1 \times J.W.$), gave all positive individuals, whereas the back-cross to the common house mouse variety gave all negative animals. Here again insufficient data were obtained. The investigators concluded that for the successful transplantation of neoplasms there must be present simultaneously in the host the factors necessary for growth. These factors are genetic determiners transmitted to the offspring through the germ-plasm. These factors may exist in the heterozygous condition, as in the F_1 hybrids, that is, Aa, Bb, Cc, etc. The particular tumor employed required the simultaneous presence of from twelve to fourteen genetic factors for the continued growth of the transplant, all of which were introduced by the Japanese waltzing stock.

Little was analyzing other tumors when an epidemic played havoc with his mouse colony, so he turned his attention to other problems. His contribution to the transplantation problem cannot, however, be over-estimated. His pioneer work served as the basis for all future genetic investigations on the transplantation of malignant tissue. He recognized the importance of using pure stock and started to inbreed his well known dilute brown stock in 1909.

Strong (1922) transplanted two adenocarcinomata arising independently of each other in two individuals of the "pure" dilute brown strain of mice and demonstrated further that susceptibility and non-susceptibility are the result of the genetic constitution of both the host and the neoplasm employed. In 1924, Strong was able to demonstrate this inter-play of genetic factors in the reaction to a sarcoma having a high degree of specificity (growing in nearly every individual inoculated), known as the Crocker Research Fund tumor No. 180. The increased growth rate observed in mixed stocks (hybrids) was explained as resulting from the manifestation of their genetic constitution. An elaboration of the above finding is essential to the present problem.

Two microscopically indistinguishable adenocarcinomata arising in two individuals of the inbred dilute brown stock were found to have different physiological characteristics or activities when inoculated in the same series of hosts (Little and Strong, 1924). Members of the parent strain of mice (the dilute browns) were all susceptible to both transplants, and all F_1 hybrids made between

the parent stock and a totally unrelated pure inbred non-susceptible race would also grow the tumors progressively. The two neoplasms reacted differently in the F_2 generation. One of the neoplasms (dBrA) grew in all F_2 individuals having the simultaneous presence of the three dominant mendelian factors necessary for growth, whereas the other (dBrB) gave a two factor ratio in the F_2 generation. With two exceptions, all mice growing the dBrA tumor also grew the dBrB tumor. Some grew the dBrB tumor but not the dBrA tumor. The observation was: $154 + B + A : 49 + B - A \pm 4.14$; the percentage negative was 24.13 per cent ± 2.04 . According to a one-factor difference ($3 + : 1 -$), the expectation was: $152.25 + B + A : 50.75 + B - A \pm 4.16$; the percentage negative: 25.00 per cent ± 2.05 . The difference between the observation and expectation was: 0.87 per cent ± 2.89 or $0.30 \times P.E.$

From this and subsequent experiments extending over a period of years, Strong and Little formulated the hypothesis that "the fate of the implanted tumor tissue when placed in a given individual (host) is brought about by a reaction between the host, determined to a large extent by its genetic constitution, and the transplanted tumor cell, controlled to some extent by its genetic constitution."

The further development of the above genetic theory of transplantation has come largely through the investigations of Strong. Most of this work will be given consideration later. Let it suffice to say, for the present, that, for the inoculation of individuals of known pedigree with various tumors, all the data secured have fallen in line with the genetic theory of transplantation as stated above. All phenomena of transplantation have been investigated and brought into line with the main genetic theory (Strong 1926d).

Evidence which Wood and Curtis believed had considerable weight against the genetic theory was accumulated by them in 1922. Using the Crocker Fund mouse tumor No. 180, they inoculated a series of mice at four different sites: right and left axilla and right and left groin. This tumor is very vicious. Of the 413 mice living two weeks after inoculation, only one was negative to all four transplants; 5 showed one growth; 20 showed two; 95 showed three; and 292 were susceptible to all four transplants. When the animals were killed at the end of the second week the tumors growing in the same individual were found to be of different sizes. From this experiment Wood and Curtis concluded "that there are probably many factors which determine

the fate of a tumor graft, and that the physiological and anatomical peculiarities of the host, which include but are not necessarily confined to its genetic constitution, are not necessarily the deciding ones."

In an experiment of this sort, it would appear unwise to use a tumor which grows as rapidly as the Crocker Fund tumor No. 180. Only individuals were employed which lived two weeks, and the probability is that the majority of these would not have lived beyond the third week following inoculation. As the total number of inoculations numbered 1,652, it is impossible to believe that faulty technic did not result in some cases. Reinoculations could not have been made, as the hosts did not survive a sufficient period following the original inoculation. These results are not in agreement with Loeb's work on rats (1902).

The author (1930) has accumulated evidence in an experiment of the above nature. Four hundred and sixty-two individuals were inoculated simultaneously in four different positions with specimens derived from the same tumor mass (dBrB). Previous work (1929) demonstrated that all individuals of the stock used were not susceptible to the neoplasm (an adenocarcinoma which arose spontaneously in the same stock about ten years ago). Of the 462 individuals inoculated, 344 showed some transplanted masses. Of the 344 animals which showed growths, 342 were susceptible to all four transplants and the remaining 2 showed three masses. Individuals which were negative in one, two, or three positions at the end of the fourth week following the initial inoculation, were reinoculated and always gave positive results.

The data also show that a larger percentage of mice showed masses in the axillary positions than in the iliac positions at the end of the third week following inoculation (65.5 per cent as compared with 52.7 per cent). By the end of the fourth week the percentages for the different positions were practically the same (73.8 per cent for the axillary inoculations and 73.4 per cent for the iliac inoculations). The growth rate for the various tumors was determined by removing the neoplasms and weighing them; that masses from the axillary positions were larger at the end of the fourth week than those which grew in the iliac region. This difference was maintained through the sixth week.

This experiment determined that a host responds consistently to multiple inoculations from the same tumor. A mouse either grows all four transplants progressively or it grows none.

The mass of evidence accumulated over a period of ten years on

thousands of inoculated animals, is, therefore, all in favor of a genetic interpretation of transplantation.

C. "*Mutations*" within *Transplanted Tumors and Pure Stocks*: Bashford (1911) observed histologic changes in tumors during the process of transplantation, which he termed mutations. As a rule, Bashford observed, the undifferentiated tumors (the cells being more embryonic or primitive in structure) grow more rapidly than the highly differentiated ones, but the differentiated tumors may exhibit as high a percentage of positive reactions as the undifferentiated.

After securing a constant reaction potential (transplantability percentage) for a tumor (dBrC), Strong (1926b) was able to demonstrate that new "physiological" types may arise during the process of transplantation. From the original mass he secured four types: "(1) the original tumor, dBrC, still giving a 5-7 mendelian factor ratio, (2) dBrCm giving a two factor ratio, (3) dBrCsp showing a one factor ratio, and (4) dBrCx growing in all mice inoculated—that is, non-specific."

To explain these various types, Strong employed the term "mutation," by which he implied "a change or shift within the genetic or internal constitution which results in definitely clear cut or discernible differences in behavior or structure that is perpetuated by a process of heredity (in this case, cell division)." Similar changes have been observed by Strong in other tumors. In every case the number of factors involved in the "mutants" has been less than for the original mass. That is, the change has always been towards more malignancy and less "tissue specificity." By means of this mutation process, it is possible to explain the variations in other experiments which the authors have attributed to rhythms, virulence, or adaptation (to be considered later).

The author (1929) has shown that changes resembling mutations may take place within a pure stock as far as the reaction to a transplanted tumor is concerned. Formerly all individuals of a given stock were susceptible to a tumor (dBrB) which arose spontaneously in a mouse of the same stock. At the present time about 20 per cent of the individuals inoculated are negative to dBrB. Whether this difference of susceptibility is confined to various sub-lines of the stock cannot be stated definitely, but such appears to be the case.

It is, therefore, highly probable that the genetic process of "mutation" may lie at the basis of changes in the constitution of

both the "host" and the "tumor-cell" component in the reaction which ensues after the placing of a bit of cancerous tissue into a series of hosts over a period of time.

D. Linkage: Strong (1929) has secured data which indicate that for the growth of a particular tumor (F_1D_g) there are probably involved the simultaneous presence of 4 mendelian factors, one of which is undoubtedly sex-linked. It has also been demonstrated by Little and Strong (1924) that the same genetic determiner may be involved in the growth of different transplants. For example, two of the three mendelian genes involved in the growth of the dBrA tumor also caused the host to be susceptible to the dBrB tumor.

Thus the problem of the transplantation of cancerous tissue is gradually being placed upon a strict genetic basis. In the last analysis, as Strong pointed out some time ago, the problem may eventually be solved by genetic methods (1926c and d).

Transplantation-susceptibility Factors

A. Race: The problem of "tissue specificity" has been referred to in every experiment discussed in the previous section where pure stocks were employed. By this term we imply that tumors arising in stocks of known genetic constitution will grow in all other individuals of the parent stock or in the first filial hybrid generation derived from mating individuals of other pure stocks to individuals of the parent race. The percentage of susceptible individuals in the F_2 generation is dependent upon the number of factors necessary for the continued growth of the tissue inoculated.

Although it has been well established that tumors arising in one species will not grow in members of another, Lambert and Hanes (1911) found that mouse tumors will grow *in vitro* in the plasma of guinea-pig, rabbit, man, and dog, but not of the goat. They concluded that the resisting agent was not in the blood. Rous and Lange (1914) demonstrated that a sarcoma arising in the Brown Leghorn would grow better in Plymouth Rocks than in the parent stock.

There appears to be quite a difference between tumors as to the rôle of race in the process of transplantation. Some tumors show a high degree of "tissue specificity"; others, like the Crocker Research Fund tumor No. 180, show very little. The latter type of transplant may be explained in terms of genetics by maintaining

that some tumors depend on host factors for their growth, which are common or fundamental to a large number of domestic races of experimental animals. There is some experimental data for this hypothesis, as recently pointed out by Strong (1926b).

Race is unquestionably a very vital factor in tumor transplantation. Its rôle can and has been analyzed in terms of genetics.

B. Age: Loeb (1901) was the first to notice a difference in susceptibility due to the age of the hosts. Transplanting a rat sarcoma, he found that immature animals and those past the sexual age were more susceptible than adults; a conclusion which has been verified by Strong (1924) on mice. Strong used an adenocarcinoma. Ehrlich and Apolant (1905) stated that age had no influence on transplantation.

Gussio (1911) demonstrated that young rats (eight to nine days) were more resistant to a sarcoma than sexually mature animals. Inoculating a sarcoma into very young mice (two to twenty days) from a non-susceptible stock, Little (1920) was able to point out that these young individuals may show temporary or progressive growth of the implant, not frequently encountered in adults.

The frequently encountered opinion regarding age is that young individuals and those past sexual maturity of a resistant or non-susceptible stock will often show masses, temporary or permanent, a condition not encountered in normal adults. *Vice versa*, the same age classes of individuals (young and old) of a susceptible stock may give negative results.

It is usually considered that mice between six and ten weeks of age are best suited for the inoculation of neoplastic tissue. This is the time of early sexual maturity, at which the complete and characteristic reaction potentialities of the species or variety are becoming manifest.

C. Pregnancy: The effect of pregnancy on transplanted neoplasms is still a matter of dispute. Morau (1891-1894) said that pregnancy accelerated their growth in both rats and mice. This theory has been supported by Herzog (1902) and Loeb (1902).

The hypothesis that pregnancy retards the growth of transplanted tumors and gives negative results has been supported by Haaland (1907), Bridre (1907), Ehrlich (1908), and Fichera (1911).

Kross (1921) claims that pregnancy has no effect on the growth of the transplanted tumor.

Cuénot and Mercier (1919) recognized three phases: (1) grafts which grew throughout pregnancy and receded during lactation; (2) grafts which continued to grow if their vascularity was independent of that of the mammary gland; (3) tumors which had remained stationary or receded during pregnancy or lactation and began to grow after lactation was over. Only six mice were used, two in each series.

More work on this problem needs to be done. The great difficulty for analysis comes from the fact that suitable controls cannot always be had. Up to the present time no standard results could be obtained. Tumors growing in "market mice" undergo peculiar changes at unpredicted times, thus leading to data which are difficult to analyze. It is hoped that with the development of pedigreed genetic stocks of experimental animals these factors underlying transplantation can be more carefully investigated.

D. Nutritional and Other Environmental Factors: Haaland (1907) and Stahr (1907) attributed some differences in susceptibility to diet. The number of animals employed by both investigators was too small to have any weight. Fleisher and Loeb (1906) stated that diet or environment has no bearing on the results secured.

That the Buffalo rat sarcoma would grow better in hosts which had been fed for several weeks on a diet containing carbohydrates (especially lactose) was claimed by Van Alstyne and Beebe (1913-14). This work has been criticized by Woglom (1915) on the ground that the sarcoma employed did not give uniform results. Repeating the experiment on both rats and mice, Woglom secured negative results.

Articles have been published by Sugiura, Benedict, and others on the effects of diet and dyestuffs on the growth of transplantable tumors. The results obtained have not been constant. The numbers of animals used have been small, 10 controls and 10 experimental animals being the greatest number employed in any one experiment, and the controls did not give uniform reactions to the transplants.

Jensen (1903) crushed the cells of the tissue prior to inoculation and did not secure growths. Bashford (1911) stated that it was necessary to determine by trial and error the optimum conditions for the transplantation of various tumors. Some transplants grow better or are more successful when emulsions are used; in other cases better results are obtained with fragments. That season has no bearing on the results was claimed by Prime (1920).

Vascularity may play an important part in the development of grafts, as was pointed out by Sittenfield (1911-12). Ligation of the artery supplying the area where the graft was placed resulted in susceptibility in 18 per cent of those inoculated, as compared with 70 per cent in the controls. Hyperemia increased the number of growths and resulted in larger tumors.

Again we notice that the conclusions arrived at by various investigators on the effects of diet and other environmental conditions are not in agreement.

E. Natural Resistance: The natural resistance (or susceptibility) of individuals to transplantable tumors has been discussed above. In order to secure constant results, however, the genetic constitution of the individuals inoculated (hosts), as well as of the individual giving rise to the tumor, must be known. The use of "market mice" will introduce all sorts of variations not encountered when pure stocks are employed.

Pitzman (1914) maintained that fluctuations in immunity may be due to bacterial contamination of either the tumor tissue employed or the immunizing material, or possibly of both.

The spontaneous regression of transplanted tumors was first mentioned by Loeb (1901). It has been encountered by nearly every investigator on transplantation. Bashford, Murray, and Bowen (1908) stated that regression may result from a limited number of growth centers causing greater homogeneity of the tumor cells. Due to fluctuations in the proliferative energy (resulting in rhythms), any single tumor cannot be regarded as being made up of cells with equal proliferative power. Tumors having cells with decreased proliferative energy would thus regress spontaneously.

Strong (1929) was able to secure a stock in which inoculated tumors consistently regressed, thus demonstrating that spontaneous regression (as well as susceptibility, *i.e.* progressive growth) of transplanted tumors may be explained in terms of genetic determiners or factors.

There is no question that resistance and susceptibility to transplanted tumors are genetic phenomena. The spontaneous regression of neoplasms has been explained on the same basis in the only investigation which has been planned to study this problem on the inoculation of pure or pedigreed stocks.

F. Acquired Resistance: Ehrlich (1907) maintained that individuals which refused to grow an implanted carcinoma were

resistant to grafts of all other types of carcinoma, as well as to all sarcomas, and that the same was true when sarcoma was employed in the original inoculation. These results are contrary to the findings of Hertwig and Poll (1907), and of Borrel (1907), and to observations made in this laboratory on pure stocks.

E. E. Jones (1926) secured growths in two naturally non-susceptible stocks following the inoculation of a small piece of undyed flannel with the tumor tissue. The resulting nodules were histologically the same as the original tissue. These "induced" tumors grew when inoculated into the original susceptible stock. The "induced" tumors were removed from the hosts not later than thirty days following inoculation. That these might have regressed is indicated from the following quotation: "On the thirtieth day after inoculation, only four of the remaining nine mice that had had masses on the eighteenth day, still had nodules."

That the flannel served as a "scaffolding," which enabled the tissue to secure a foothold, cannot be denied, but the final results were not stated. Progressive growth, at least, did not occur.

Temporary growth of albino tumors in a totally unrelated non-susceptible stock has been encountered by Cloudman (unpublished) without the aid of an irritating agent.

As with many of the other problems on transplantation, the status of acquired resistance needs further investigation. Whether the temporary growth of a tumor in a naturally non-susceptible stock is the result of induced materials or a natural condition, cannot be stated.

Many agents (physical, chemical, biological) have been used by a large number of investigators in order to induce immunity to a transplanted tumor. A list of these would cover practically the entire number of known variants. Conflicting results have ever been the outcome. No uniformity has been encountered. One explanation of this situation has been offered by Woglom (1929), based on work published by Russell (1912), that propagable tumors may be divided into two groups, one series which immunizes the host and one which does not.

Another possible explanation is that the problem lies in another field of human knowledge, genetics. The methods of attack of this science have not been taken into consideration. Where genetic methods are recognized and utilized, uniformity of results has ever been obtained.

Fluctuation of the Tumor Cell

A. Rhythms: The English school, headed by Bashford, has published numerous articles explaining the variations in susceptibility in the various generations of transplantation as due to rhythms or fluctuations within the tumor cell (1905, 1907, etc.). The different tumors give maximum numbers of takes followed by a great decrease in the percentage of growths, succeeded in turn by an increase to a new maximum. To their own satisfaction these investigators proved that "the conclusion appears justified that the power of establishing themselves in new hosts varies periodically in these tumour cells from inherent causes" (1907).

Another view of the question is held by Strong (1922, 1929). In 1929, he wrote: ". . . rhythm of physiological activity on the part of the tumor cell is an artifact and does not exist in nature . . . by careful study of genetic transmission of susceptibility or immunity, determined by means of genetic analysis, one is enabled to demonstrate that *every* mouse responds to this tumor (either by tolerance or by refusal to harbor the transplant) because it has received its proper genetic constitution from its direct ancestry."

As Strong's work was done with pure stocks (or with mice of known ancestry), it is easy to understand why his results differ from those of Bashford and his associates.

B. Virulence or Adaptation: There are two theories regarding the increase in the number of growths for successive generations following the transplantation of spontaneous cancer. The first, termed the virulence theory, has been supported by Ehrlich and Apolant; the second, or adaptation theory, has been advanced by the English group of investigators (Bashford, Murray, Cramer, Haaland, and Bowen), and by Woglom in America.

Ehrlich (1907) believed that tumors with an increased virulence could be obtained by transplanting the more rapidly growing tumors in each series. His results were not constant, as not every strain gave tumors having an increased growth capacity.

Apolant (1909) recognized two factors for virulence: (1) transplantability, determined by the number of susceptible takes, and (2) proliferative energy, based on the rate of growth. Apolant also found exceptions to his theory that slow-growing tumors gave few growths and rapidly growing ones numerous successful transplants.

In support of the other theory, Bashford and his associates (1908) arrived at the conclusion that the failure of the original

tumor to grow was due to the inability of the transplanted cells to adapt themselves to the new conditions existing in the host. Adaptation of the tumor cells, resulting in the increase in positive takes, was observed following continued transplantation.

In the application of the "mutation" process, Strong has attempted to explain this phenomenon in another way. He has found that many transplanted tumors mutate, that is, they start giving a relatively high mendelian ratio in the segregating F_2 generation (produced by originally crossing two individuals of two totally contrasting strains, one of which is 100 per cent susceptible to the transplant, the other one 100 per cent resistant); then after a time they "break down," giving a lower mendelian ratio in the same series of animals. This process of "mutation" has always been towards less "tissue specificity," that is, permitting the tumor to grow in more individuals and at a greater rate. The "mutation" has (1) changed "the reaction potentiality" of the tissue in the host; (2) its transplantability factor, and (3) the proliferative energy capacity. Mutations may also be said to underlie the process of tissue adaptation, as maintained by Bashford in England and Woglom in America. The theory of mutation, therefore, clears up the conflicting conceptions encountered under "virulence" or "adaptation" on the part of the tumor cell during the process of transplantation.

Genetic Theory of Transplantation

By the above statement one may arrive at one of two theories regarding the transplantation of neoplasms. The first, advanced by the English group of investigators (Bashford, Murray, Haaland, Bowen, and Cramer), and supported by a few workers, as Woglom, in America, maintains that the tumor plays the more important rôle in transplantation, with the activity of the host in a secondary position. To demonstrate this theory its advocates have advanced results such as rhythm, adaptation, or increased virulence of the tumor cell.

The second or genetic theory of transplantation has been advanced by Tyzzer, Little, and Strong, and has the support of Loeb, although his interpretation differs from that of the others. The substance of this genetic theory is that susceptibility to transplanted tumors depends on the simultaneous presence of certain dominant mendelian factors in the fundamental make-up in the host. The number involved may and does vary for different

tumors, and can only be determined in the F_2 and back-cross generations by a comparison between the observation and expectation for various factors. Furthermore, these mendelian units need be present only in the heterozygous condition to insure susceptibility (as is encountered in the F_1 generation). Individuals (hosts) lacking one or more of the necessary growth factors show spontaneous regression or are resistant to the transplant.

Strong maintains, as a result of a careful analysis of the genetic constitution of the different stocks and generations, that rhythms do not actually exist. Virulence or adaptation may also be explained by a process analogous to somatic mutations in the intrinsic constitution of the tumor cell.

It is not hard to understand why this difference of opinion exists. The data on which the first theory was based were secured from the inoculation of individuals with unknown pedigrees, in other words, "market mice." The investigators holding the theory are mostly men who have not been trained in the methods of genetics. The second theory is based on data secured from the inoculation of pure stocks or hybrids of known ancestry by geneticists, and uniform results have been secured.

A. Problems Investigated: Very little work has been completed on the problem of the inoculation of neoplastic tissue, although volumes have been written. Of the three well known inbred stocks of mice (albino, dilute brown, and Japanese waltzing), only the dilute brown has received careful consideration. The natural characteristics of the waltzing stock makes it an unsuitable race for extensive investigation. Cloudman is using albino tumors in his investigation, which will add materially to the present data.

The field opened by mutational changes occurring within the tumor cell during the process of transplantation has only been touched upon. Only one example of linkage has been reported (Strong, 1929).

B. Problems to be Investigated: The most important problem which should receive careful attention in the near future is that of linkage. Not until linkage has been thoroughly demonstrated will the problem of tumor transplantation be acknowledged as a proved genetic problem. The present investigation was started with that end in view, but the loss of the tumor tissue employed has caused delay. New tumors have already been inoculated to carry out the original purpose. In order to analyze the data to observe the presence of linkage, should it occur, the hybrid and

back-cross generations will be made by all possible matings, crosses which are impossible at present because of lack of facilities.

Apart from the present investigation no work has been completed on the transplantation of neoplasms arising in hybrids from inbred strains. That the results are in agreement with the genetic theory of transplantation will soon be evident.

Another problem upon which we hope to start work within the near future is the inoculation of tumors (should they occur) in hybrids made by crossing an inbred race, which gives rise to a large number of spontaneous cancers, with another relatively inbred stock which is a very low tumor line.

"Mutational" changes within tumors will receive their share of investigation. Along this same line will be an investigation of the changes in Murray's dilute brown stock, which have altered the susceptibility of these mice to a dilute brown tumor. Sufficient mice (over 1,000) have already been inoculated to serve as a guide for further work.

The problems that have been investigated in this present paper may be stated as follows:

1. Genetic analysis of two tumors arising spontaneously in the same mouse.
2. Genetic analysis of tumors arising in an F_1 individual whose genetic constitution is heterozygous for a large number of genetic factors.
3. Further analysis of the "mutation" process during the transplantation of tumor tissue.
4. The physiological characteristics of the individual as controlled by genetic factors.
5. The fundamental nature of the organism.

The author is greatly indebted to Doctor C. C. Little, who has made this experiment possible. The problem was suggested and the manuscript was written under the personal direction of Professor L. C. Strong. For the photography acknowledgment is due Doctor J. M. Murray and Doctor W. S. Murray.

EXPERIMENTAL STUDY

Materials

A. Races Employed: To fulfil the requirements for the problem at hand we have used two parent stocks of highly inbred mice. The albino stock was originally received from Dr. H. J. Bagg, of Memorial Hospital, New York City. He has inbred this strain,

brother-to-sister matings, since 1912. A derivative of the original stock was secured by Dr. Strong in 1918 and has been continued by like matings since that time. This race of mice has received the designation "A," which will be employed to distinguish it in the following pages.

The inbreeding of the dilute brown stock, the other parent strain, was started by Dr. Little in 1909. The individuals used in the present experiment were from Strong's derivative of this

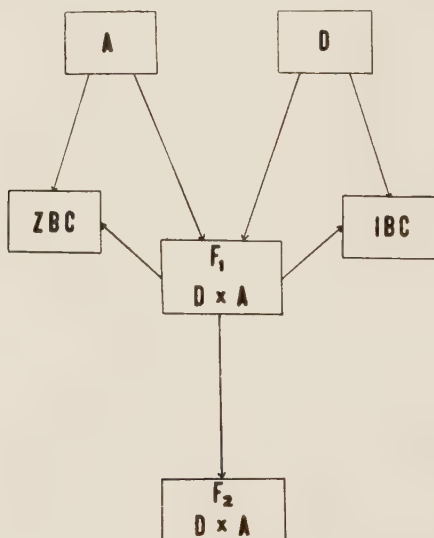


FIG. 1. THE RELATIONSHIP EXISTING BETWEEN THE VARIOUS STOCKS AND GENERATIONS OF MICE EMPLOYED

The two parent inbred stocks are called "A" (albino) and "D" (dilute brown). Two arrows pointing towards a square indicate that the particular generation was produced by crossing the two stocks or generations from which the arrows come. One arrow indicates that the matings were *inter se* to produce the next generation.

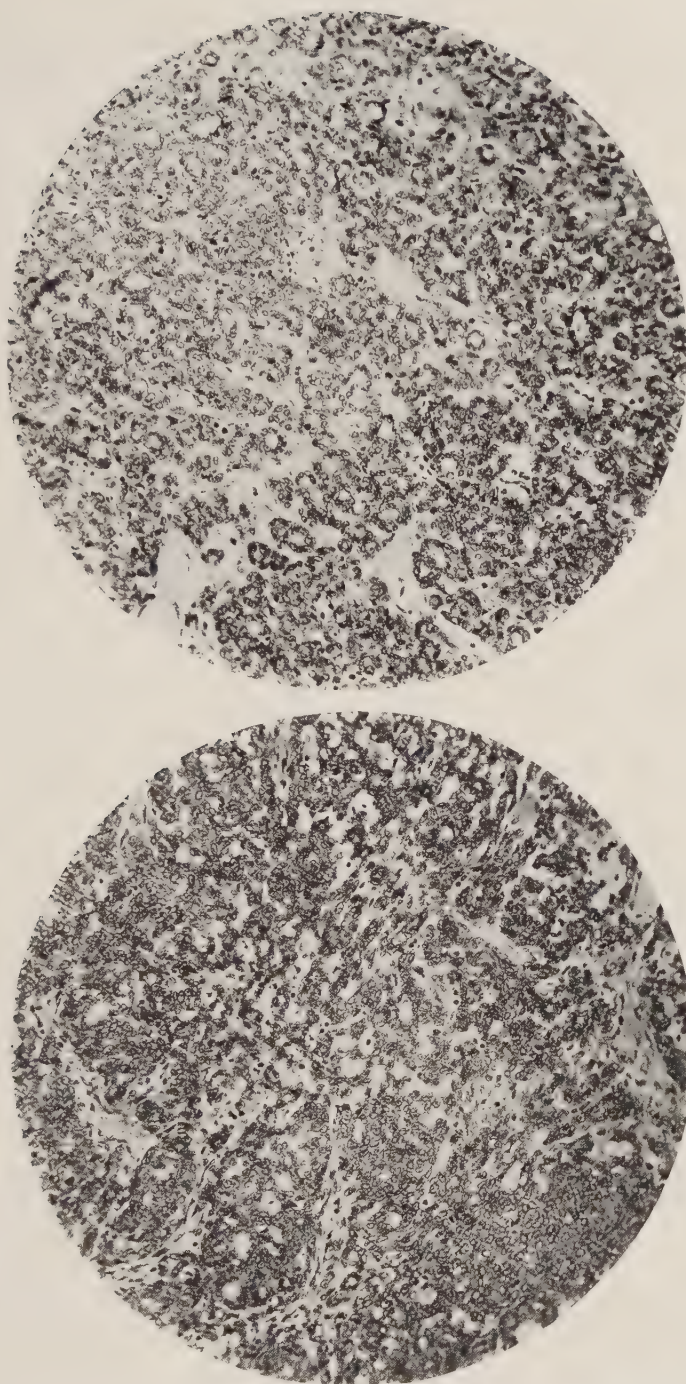
stock, which have been separated from Little's stock since 1921. The dilute brown race has been called by us the "D" stock.

The first filial (F_1) generation was made by mating A females with D males (Fig. 1). F_1 's made in the reciprocal direction have not been included in the present problem.

The second filial (F_2) generation was made by mating F_1 individuals *inter se*.

The back-cross generation ZBC resulted from crossing F_1 females with A males. The other back-cross generation, IBC, was made by crossing F_1 females with D males.

All mice were kept in wire cages made of heavy one-fourth



FIGS. 2 AND 3. PHOTOMICROGRAPHS OF TUMOR 13714B (OR B) AND TUMOR 13714A (OR A), BOTH DIAGNOSED AS ADENOCARCINOMA AND HISTOLOGICALLY INDISTINGUISHABLE. $\times 375$

inch wire mesh. Two cages are placed in a tin pan upon a layer of wood shavings. The cages are cleaned once a week and sterilized about every three months.

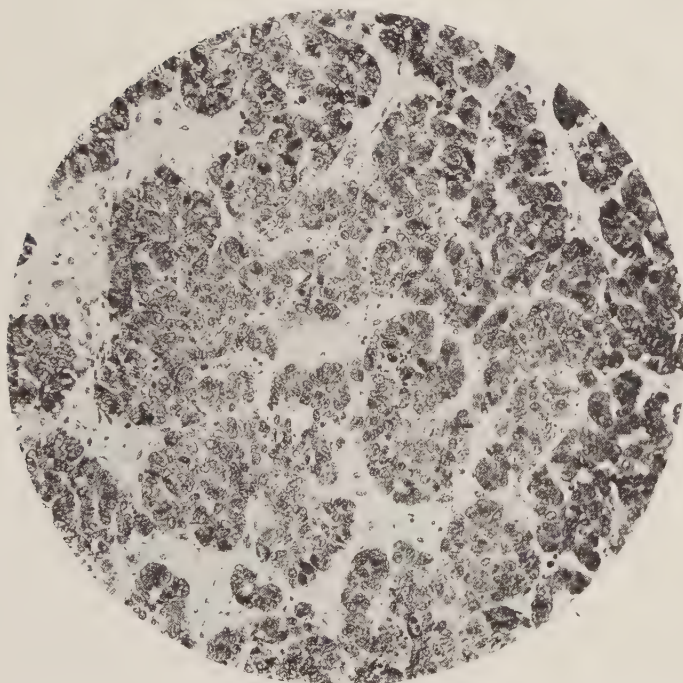
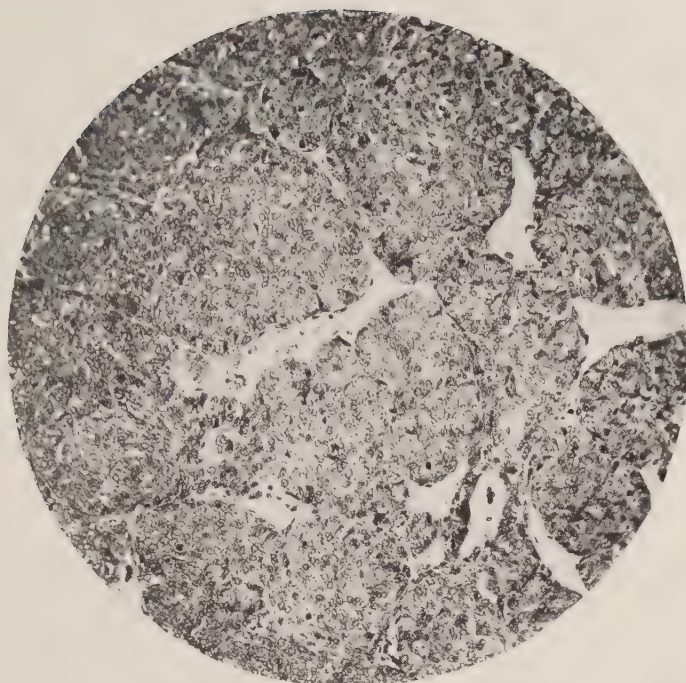
The main food for the mice is a mixture of rolled oats, fine meat scraps, powdered milk, and salt. Once a week the mice are fed on grain (hemp and canary). Dog biscuit is always available. The use of this diet over a long period of time has shown its complete adequacy.

At the time the young are weaned they are given serial numbers. Identification markings are made in the ears by a series of holes and notches. The unit digits are represented on the right ear and the ten digits on the left ear. The complete serial numbers for the mice are kept on individual cards.

B. Tumors Employed: The two tumors originally employed arose spontaneously and independently of each other in an F_1 female, No. 13714. Both masses were recorded the same day, April 4, 1928. The larger of the two, which we have called tumor No. 13714A (or A), occurred in the dorsal neck region. The other, tumor No. 13714B (or B), was observed in the ventral neck region. The animal was killed on May 5, 1928, and the tumors inoculated into several individuals. Sections of the masses were saved for histologic examination. Both tumors were diagnosed² as adenocarcinomata and were histologically indistinguishable. Figs. 2 and 3 are photomicrographs of the original tumors, 13714B (or B) and 13714A (or A).

During the process of transplantation the two tumors gave constant reactions for several transplant generations when inoculated into F_2 individuals (determined by calculating the percentage of susceptible individuals in the various experiments). After the tumor 13714A had been transplanted for four transfer generations and the tumor 13714B for five, very noticeable differences were observed in the percentage of susceptible individuals. Other differences were noticed which will be discussed later. The symbol for the tumor 13714A (or A) was at that time changed to 13714AX (or AX) and for the tumor 13714B (or B) to 13714BX (or BX). The two tumors 13714BX and 13714AX were found to be more cellular in type but the histologic structure of all four tumors was identical. Figs. 4 and 5 are photomicrographs of tumors 13714BX and 13714AX.

² The author is greatly indebted to Dr. A. S. Warthin for the diagnosis and comparison of the different neoplasms.



FIGS. 4 AND 5. PHOTOMICROGRAPHS OF TUMOR 13714BX (OR BX) AND TUMOR 13714AX (OR AX), BOTH DIAGNOSED AS ADENOCARCINOMA AND INDISTINGUISHABLE FROM TUMORS 13714B AND 13714A. $\times 375$

Methods

A. Inoculation: The trocar method of inoculation, as described by Strong (1922), was used throughout the investigation. Only ordinary aseptic precautions are required.

B. Observation and Measurement: Three weeks after inoculation or reinoculation, and then weekly until the eighth week, the mice were examined for the presence of tumors by palpation. The observation data were recorded on coordinate paper.

C. Condition of Transplantation-susceptibility Factors: In order to clear up any misunderstanding regarding the much disputed transplantation-susceptibility factors, a paragraph should be given on their effects, should they occur, on the final results.

All individuals of the F_2 , 1BC, and ZBC generations (the important ones for the determination of the number of dominant mendelian factors present) were inoculated between the ages of seven and ten weeks. Some mice of the F_1 generation and of the A and D stocks were older than ten weeks when employed. It had, however, previously been proved that the age factor did not influence their reaction to the implant. At the time the young of the F_2 , 1BC, and ZBC generations were weaned, they were separated according to sex when placed in cages. An average of eight mice were put in a cage. The influence of pregnancy, if any does exist, was not present for these classes. In the other stocks (F_1 , A and D) some of the females undoubtedly were pregnant at the time of inoculation or became so soon afterward.

The nutritional and other environmental factors were the same for all the stocks and generations. All the mice were housed in one large room.

Results

A. Inoculation of Tumor 13714B (or B): The data for the inoculation of tumor 13714B (or B) will first be given. This tumor was always inoculated in the *left* axillary position and transplanted simultaneously, with a few exceptions, with either tumor 13714A (or A) or tumor 13714AX (or AX). The A or AX tumor was always inoculated near the *right* axilla.

The observation data for all the tumors appear in Table I.

One hundred and nineteen individuals of the F_1 generation were inoculated with tumor 13714B; of these, 118 grew the tumor. Three weeks after inoculation 117 were growing the grafts. Of the remaining 2 mice, 1 grew the tumor upon reinoculation, while the

other proved to be negative (—). This mouse which was negative was reinoculated without success. As it was growing tumor 13714A very rapidly, the animal had to be killed before it could be reinoculated a second time.

For the sake of clarity we have presented the original data in the form of curves where such a procedure is possible. In the determination of the curve representing the number of individuals growing the tumor inoculated, we have included in the percentage susceptible (+) all mice showing masses for the stated week, regardless of whether the tumor later regressed or continued to grow. The number of susceptible mice (for the curve) may thus

TABLE I

Results Secured from Inoculation of Different Stocks and Hybrid Generations with Various Tumors

Stocks and Hybrids	Tumor 13714B (or B)			Tumor 13714A (or A)		
	+	—	Per Cent Negative	+	—	Per Cent Negative
F ₁	118+	1—±0.66	0.84%±0.55	121+	0—	0.00%
D	3+	177—±1.15	98.33%±0.64	3+	141—±1.13	97.92%±0.65
A	0+	132—	100.00%	0+	32—	100.00%
F ₂	11+	262—±2.17	95.97%±0.79	9+	203—±1.93	95.75%±0.92
1BC	1+	21—±0.65	95.45%±3.05	3+	19—±1.07	86.36%±4.96
ZBC	2+	66—±0.93	97.06%±1.38	0+	16—	100.00%
	Tumor 13714BX (or BX)			Tumor 13714AX (or AX)		
	+	—	Per Cent Negative	+	—	Per Cent Negative
F ₂	94+	250—±5.51	72.67%±1.57	61+	344—±4.80	84.94%±1.19
1BC	23+	44—±2.59	65.67%±3.87	14+	53—±2.20	79.10%±3.31
ZBC	34+	434—±3.74	92.74%±0.81	13+	507—±2.37	97.50%±0.46

differ from the third through the seventh weekly observation period. As all mice in the various generations in which tumors regressed showed this by the end of the seventh week, the number given for the “+” period represents the actual number of susceptible individuals (Table I).

In case mice were reinoculated and showed masses by the end of the third week following reinoculation, they were included as susceptible in the third weekly period. The reason for this is that the failure of the host to grow the transplant after the initial inoculation was probably due to faulty technic.

Since one of the 2 F₁ individuals which were reinoculated

showed a mass by the end of the third week following reinoculation, it was included under the third weekly period as susceptible. The percentage of susceptible mice for the F_1 generation thus remained constant from the third through the seventh and “+” weeks. The curve is given in Fig. 6.

The probable errors are: $118 \pm 1 - \pm 0.66$; the percentage negative: $0.84\% \pm 0.55$.

The 36 individuals of Strong's derivative of the D stock which were inoculated with tumor 13714B were all negative. One hundred and forty-four mice belonging to Murray's derivative of

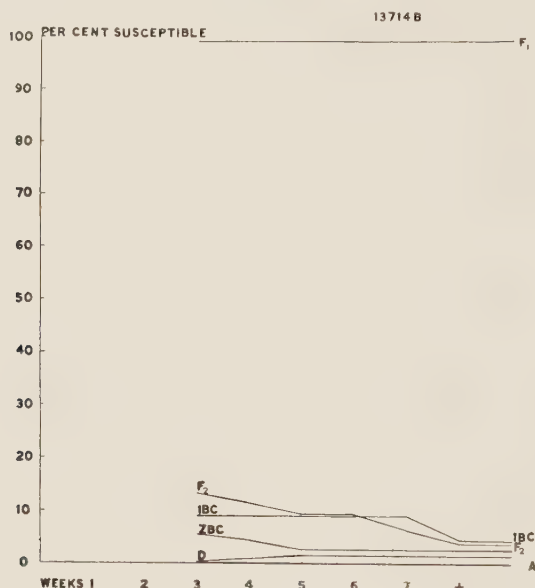


FIG. 6. CURVES REPRESENTING THE PERCENTAGE OF SUSCEPTIBLE INDIVIDUALS IN THE VARIOUS STOCKS AND GENERATIONS WHEN INOCULATED WITH TUMOR 13714B (OR B)

the dilute brown stock formerly belonging to Little were also inoculated, of which 3 were susceptible.³ The complete tabulations, combining the entire number inoculated from both derivatives, are given in Table I and the curve in Fig. 6.

All individuals of the A stock were negative to grafts of the tumor 13714B (or B); 132 mice were used.

Two hundred and seventy-two F_2 mice were inoculated with tumor 13714B (or B) before the “mutation” in that tumor

³ It should be remembered that the author (1929) demonstrated that not all of Murray's stock were susceptible to a spontaneous dilute brown tumor which originally grew in all individuals.

occurred. Of this number, 11 were susceptible and 25 showed temporary nodules. All the negative mice were reinoculated. In the determination of the final number negative all individuals were considered to be so unless they grew the grafts progressively. The probable errors are calculated from the percentage representing this number of negative mice. The observation for the F_2 generation was: $11 + : 262 - \pm 2.17$; the percentage negative: $95.97\% \pm 0.79$. The curve is given in Fig. 6.

The number of individuals of the 1BC and ZBC generations inoculated was too small to be of any value (Table I).

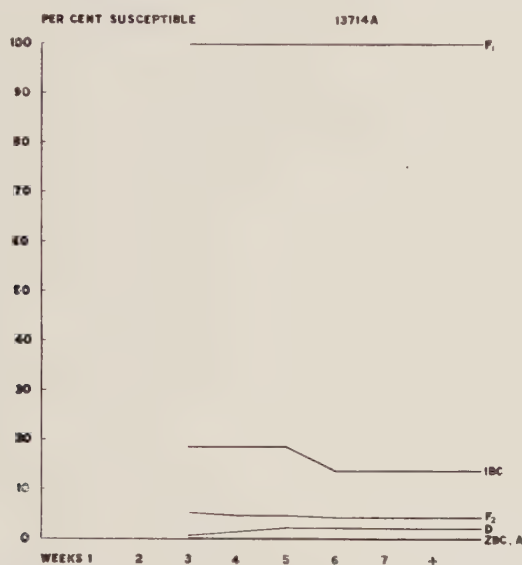


FIG. 7. CURVES REPRESENTING THE PERCENTAGE OF SUSCEPTIBLE INDIVIDUALS IN ALL THE STOCKS AND GENERATIONS WHEN INOCULATED WITH TUMOR 13714A (OR A)

B. Inoculation of Tumor 13714A (or A): With few exceptions, all the F_1 individuals which were inoculated with tumor 13714B were also inoculated simultaneously with tumor 13714A. One hundred and twenty-one animals were employed, all of which had grown the grafts by the end of the third week following the initial inoculation or reinoculation (Table 1); 4 mice had to be reinoculated. The results were: $121 + : 0 -$; the percentage negative: 0.00 per cent.

All the animals of the dilute brown stock which were inoculated with tumor 13714A were of Murray's derivative of this race. One hundred and forty-four mice were employed, one of which

showed progressive growth after the first observation period and 2 following the fifth week after inoculation. The tabulations were: $3 + : 141 - \pm 1.13$; the percentage negative: $97.92\% \pm 0.65$.

All individuals of the A stock were resistant to transplants of tumor 13714A; 32 animals were inoculated.

Transplants from tumor 13714A were inoculated into 212 F_2 individuals. Of this number, the tumors spontaneously regressed in 2, while 9 showed progressive growth. The results were: $9 + : 203 - \pm 1.93$; the percentage negative: $95.75\% \pm 0.92$.

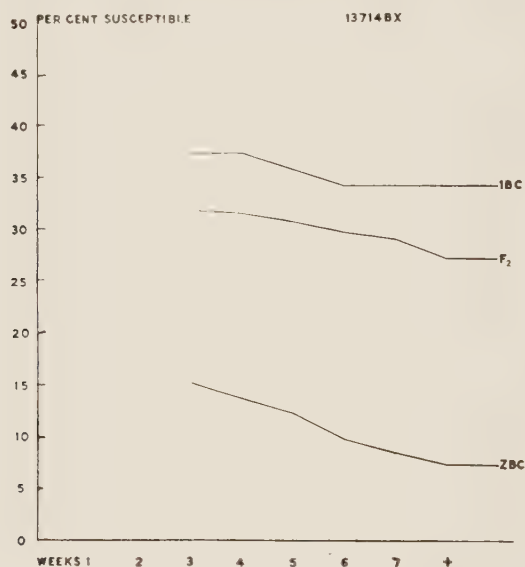


FIG. 8. CURVES REPRESENTING THE PERCENTAGE OF SUSCEPTIBLE INDIVIDUALS IN THE F_2 , 1BC, AND ZBC GENERATIONS WHEN INOCULATED WITH TUMOR 13714BX (OR BX)

The number of individuals of the 1BC and ZBC generations which were inoculated with tumor 13714A were insignificant, as in the case of tumor 13714B (Table I).

C. Inoculation of Tumor 13714BX (or BX): After the "mutation" in tumor 13714B (or B), it was designated tumor 13714BX (or BX). The determination of the exact time when this took place was made by computing the percentage of susceptible individuals in all the experiments in which F_2 individuals had been inoculated. On comparing these percentages in the various experiments, it was noticed that there had been a significant increase in the percentage of mice growing the tumor. This

increase remained constant for the remainder of the time that the tumor was transplanted.

Since the mice belonging to the A and D stocks and the F_1 generation represent inbred lines or hybrids, their reactions should be constant for the particular race or generation, when inoculated with the tumor employed. For this reason we have recorded all animals as having been inoculated with tumor 13714B, even if tumor 13714BX was employed.

Tumor 13714BX (or BX) was inoculated into 344 mice repre-

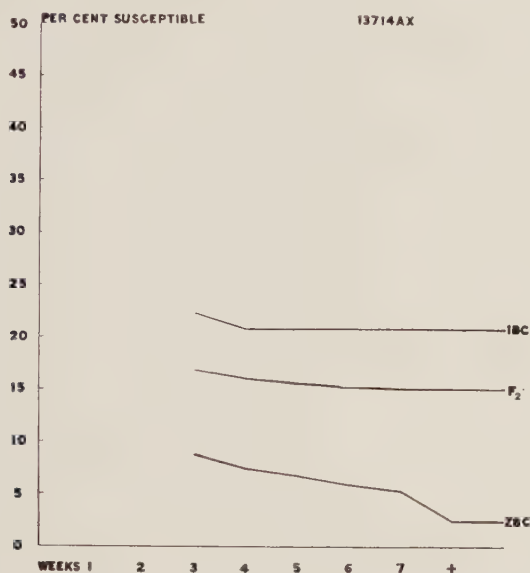


FIG. 9. CURVES REPRESENTING THE PERCENTAGE OF SUSCEPTIBLE INDIVIDUALS IN THE F_2 , 1BC, AND ZBC GENERATIONS WHEN INOCULATED WITH TUMOR 13714AX (OR AX)

senting the F_2 generation. Out of this number 94, or 27.3 per cent, grew the tumor progressively and in 19, or 5.5 per cent, the transplants regressed. The results were: $94 \pm : 250 - \pm 5.51$; the percentage negative: $72.67\% \pm 1.57$ (Table I). The curve is given in Fig. 8.

It is a matter of regret that we were able to inoculate only 67 1BC individuals with transplants from the tumor 13714BX before the tissue became infected. At that time we had over 200 young ready to be inoculated but no grafts to transplant. Of the animals employed, 23 were susceptible while 2 showed regression. The observations were: $23 \pm : 44 - \pm 2.59$; the percentage negative: $65.67\% \pm 3.87$.

Grafts from the tumor 13714BX were inoculated into 468 ZBC individuals; 37, or 7.9 per cent, showed spontaneous regression. The final observations were: $34 + : 434 - \pm 3.74$; the percentage negative: $92.74\% \pm 0.81$ (Table I).

D. Inoculation of Tumor 13714AX (or AX): Individuals which were inoculated with tumor 13714AX of the F_1 generation, A and D stocks, are included under the results secured from the inoculation of the tumor 13714A in the particular generation or stock involved.

Among 405 animals of the F_2 generation which were inoculated with grafts of tumor 13714AX (or AX), the transplants spontaneously regressed in 7, or 1.7 per cent. The final observations were: $61 + : 344 - \pm 4.80$; the percentage negative: $84.94\% \pm 1.19$ (Table I). The curve is represented in Fig. 9.

One, or 1.5 per cent, of the 67 animals of the 1BC generation inoculated with tumor 13714AX showed regression of the grafts. The other results were: $14 + 53 - \pm 2.20$; the percentage negative: $79.10\% \pm 3.31$.

The results for the ZBC generations secured from the inoculation of the tumor 13714AX were: $13 + : 507 - \pm 2.37$; the percentage negative: $97.50\% \pm 0.46$; 32, or 6.2 per cent, showed spontaneous regression.

Comparison of Observation with Expectation

According to the genetic theory of transplantation, based on the work of Tyzzer, Little, and Strong, individuals (hosts) having simultaneously in their genetic make-up the number of dominant mendelian factors necessary for the progressive growth of a transplanted tumor will be susceptible to grafts of that neoplasm. As stated above, these factors need be present in only the heterozygous condition for susceptibility; thus, they must be dominant factors. For example, if susceptibility to a transplanted neoplasm depended upon the simultaneous presence of two genetic factors, as A and B, all individuals having the following genetic constitutions would grow the transplants progressively: AABB, AaBB, AABb, and AaBb. The animals lacking one or more of the necessary dominant factors would be negative, as AAbb, Aabb, aaBB, aaBb, and aabb. When a number of factors are involved, individuals lacking one (or more?) of the dominant factors necessary for progressive growth may show temporary growth of the graft, followed by spontaneous regression.

The number of dominant mendelian factors necessary for the progressive growth of a transplanted tumor can be calculated from the results secured from the inoculation of F_2 individuals, derived originally from pure stocks giving constant reactions to the transplants. The observed results in the F_2 generation are compared with the expectation for all the possible combinations of dominant factors for this generation. The difference between the observation and expectation is determined, and the probable errors calculated. In this way, the probable number of dominant mendelian factors necessary for susceptibility to a tumor may be

TABLE II

Expected Proportions of Susceptible (+) and Non-susceptible (−) Individuals in the F_2 Generation, According to the Theory That for the Continued Growth of the Transplanted Tumor There Must be Present in the Host One or More Definite Genetic Factor (Dominance for Susceptibility Always Assumed—Based on Previous Work of Tyzzer, Little, and Strong)

Number of Factors	Factorial Composition of F_1	Expectation in the F_2 Generation	Ratio in F_2	Per Cent Negative in F_2
1	Aa	3+ : 1−	1+ : 0.33−	25.00
2	AaBb	9+ : 7−	1+ : 0.78−	43.75
3	AaBbCc	27+ : 37−	1+ : 1.37−	57.81
4	AaBbCcDd	81+ : 175−	1+ : 2.16−	68.36
5	AaBbCcDdEe	243+ : 781−	1+ : 3.21−	76.27
6	AaBbCcDdEe++	729+ : 3,367−	1+ : 4.62−	82.20
7	AaBbCcDdEe++	2,187+ : 14,197−	1+ : 6.49−	86.65
8	AaBbCcDdEe++	6,561+ : 58,975−	1+ : 8.99−	89.99
9	AaBbCcDdEe++	19,683+ : 242,461−	1+ : 12.32−	92.49
10	AaBbCcDdEe++	59,049+ : 989,527−	1+ : 16.75−	94.37
11	AaBbCcDdEe++	177,147+ : 4,017,157−	1+ : 22.68−	95.78
12	AaBbCcDdEe++	531,441+ : 16,245,775−	1+ : 30.57−	96.83
13	AaBbCcDdEe++	1,594,323+ : 65,514,541−	1+ : 41.09−	97.62
14	AaBbCcDdEe++	4,782,969+ : 257,275,087−	1+ : 53.78−	98.17

estimated, provided a large number of individuals has been inoculated and there are not too many factors involved. Factorial combinations are eliminated where the degree of significance between the observations and expectations is greater than $3 \times \text{P.E.}$

The expectations for the F_2 generation are determined in Table II for one to fourteen dominant mendelian factors. Both the actual number of susceptible (+) and non-susceptible (−) animals and the ratio of susceptible to non-susceptible individuals are given in the F_2 generation. The percentage of negative mice for the various numbers of factors has also been computed. Simultaneous presence of all dominant mendelian factors is also

assumed, based upon the pioneer work of Tyzzer, Little, and Strong.

For Tumor 13714B (or B): From the inoculation of tumor 13714B (or B) into F_2 individuals we secured the following observation: $11 + : 262 - \pm 2.17$, the percentage negative was $95.97\% \pm 0.79$ (Table I). This observation for tumor 13714B is compared with the expectation for 8, 11, and 14 dominant mendelian factors in Table III (Ratios 1 to 4), based on the calculation given in Table II.

TABLE III

Comparison of the Observed Data for the F_2 Generation with the Expectation for 8, 11, and 14 Dominant Mendelian Factors for Tumor 13714B (Ratios 1 to 4) and for Tumor 13714A (Ratios 5 to 8)

Ra- tio	Tumor 13714B	Number of Individuals	Per Cent Negative	Difference Between Ratios
1	Observation	$11.00 + : 262.00 - \pm 2.17$	$95.97\% \pm 0.79$	
2	Expectation 8 Factors	$27.33 + : 245.67 - \pm 3.31$	$89.99\% \pm 1.21$	1 and 2 = 5.98% ± 1.44 or $4.15 \times \text{P.E.}$
3	Expectation 11 Factors	$11.53 + : 261.48 - \pm 2.11$	$95.78\% \pm 0.78$	1 and 3 = 0.19% ± 0.78 or $0.24 \times \text{P.E.}$
4	Expectation 14 Factors	$4.98 + : 268.02 - \pm 1.56$	$98.17\% \pm 0.57$	1 and 4 = 2.20% ± 0.97 or $2.37 \times \text{P.E.}$
	Tumor 13714A			
5	Observation	$9.00 + : 203.00 - \pm 1.93$	$95.75\% \pm 0.92$	
6	Expectation 8 Factors	$21.22 + : 190.78 - \pm 2.91$	$89.99\% \pm 1.37$	5 and 6 = 5.77% ± 1.65 or $3.80 \times \text{P.E.}$
7	Expectation 11 Factors	$8.95 + : 203.05 - \pm 1.95$	$95.78\% \pm 0.86$	5 and 7 = 0.02% ± 1.26 or $0.02 \times \text{P.E.}$
8	Expectation 14 Factors	$3.87 + : 208.13 - \pm 1.30$	$98.17\% \pm 0.61$	5 and 8 = 2.41% ± 1.10 or $2.18 \times \text{P.E.}$

A comparison between the observation and the expectation for 8 factors gives a difference in the percentage of negative individuals of $5.98\% \pm 1.44$ or $4.15 \times \text{P.E.}$ As this difference is significant, 8 factors may be eliminated as the number involved for susceptibility to this tumor. All the other comparisons must be considered, as in no case is the probable error greater than 3. The smallest probable error, 0.24, is for 11 factors. Thus we may say that the number of dominant mendelian factors necessary for the progressive growth of the tumor 13714B (or B) is from 9 to 14, the probable number being 11.

For Tumor 13714A (or A): The observation secured from the transplantation of the tumor 13714A (or A) in F_2 individuals was: $9 + : 203 - \pm 1.93$; the percentage negative: $95.75\% \pm 0.92$ (Table I).

Table III, Ratios 5 to 8, gives the comparison between the observed results and the expectation for 8, 11, and 14 factors. Eight factors, as the probable number involved as necessary for susceptibility to tumor 13714A, may be eliminated, as the degree of significance between the observation and expectation is $3.80 \times \text{P.E.}$ Comparisons for the other factors give probable errors which are not significant.

Tumor 13714A (or A) may thus be said to require the simultaneous presence of from 9 to 14 dominant mendelian factors for

TABLE IV

Comparison of the Observed Data for the F_2 Generation with the Expectation for 4 and 5 Dominant Mendelian Factors for Tumor 13714BX (Ratios 1 to 3); for the 1BC Generation with the Expectation for 3 or 4 Factors Being Brought into the Cross by the D Stock (Ratios 4 to 6), and for the ZBC Generation with the Expectation for 1 or 2 Factors Being Brought into the Cross by the A Stock (Ratios 7 to 9)

Ratio	Tumor 13714BX	Generation	Number of Individuals	Per Cent Negative	Difference Between Ratios
1	Observation	F_2	$94.00+ : 250.00 - \pm 5.51$	$72.67\% \pm 1.57$	
2	Expectation 4 Factors	F_2	$108.86+ : 235.14 - \pm 5.75$	$68.35\% \pm 1.67$	1 and 2 = 4.32% ± 2.29 or $1.88 \times \text{P.E.}$
3	Expectation 5 Factors	F_2	$81.71+ : 262.29 - \pm 5.27$	$76.25\% \pm 1.53$	1 and 3 = 3.58% ± 2.19 or $1.63 \times \text{P.E.}$
4	Observation	1BC	$23.00+ : 44.00 - \pm 2.59$	$65.67\% \pm 3.87$	
5	Expectation 3 Factors	1BC	$16.75+ : 50.25 - \pm 2.36$	$75.00\% \pm 3.52$	4 and 5 = 9.33% ± 5.22 or $1.79 \times \text{P.E.}$
6	Expectation 4 Factors	1BC	$33.50+ : 33.50 - \pm 2.73$	$50.00\% \pm 4.07$	4 and 6 = 15.67% ± 5.61 or $2.79 \times \text{P.E.}$
7	Observation	ZBC	$34.00+ : 434.00 - \pm 3.74$	$92.74\% \pm 0.81$	
8	Expectation 1 Factor	ZBC	$29.25+ : 438.75 - \pm 3.49$	$93.75\% \pm 0.75$	7 and 8 = 1.01% ± 1.10 or $0.92 \times \text{P.E.}$
9	Expectation 2 Factors	ZBC	$58.50+ : 409.50 - \pm 4.77$	$87.50\% \pm 1.02$	7 and 9 = 5.24% ± 1.30 or $4.03 \times \text{P.E.}$

susceptibility in a given host. The probable number may be 11 factors, as the degree of significance in this case is only $0.02 \times \text{P.E.}$

For Tumor 13714BX (or BX): From the inoculation of tumor 13714BX (or BX) in the F_2 generation, the following observation was secured: $94 + : 250 - \pm 5.51$; the percentage negative was: $72.67\% \pm 1.57$ (Table I).

Comparisons are made in Table IV (Ratios 1 to 3) with the

expectation for the probable number of factors which may be involved. The degree of significance for a comparison between the observation and the expectation for 3 factors is $6.31 \times \text{P.E.}$; for 4 factors, $1.88 \times \text{P.E.}$; for 5 factors, $1.63 \times \text{P.E.}$; and for 6 factors, $4.55 \times \text{P.E.}$ The probable number of mendelian factors is either 4 or 5, probably 5.

Following the determination of the number of factors necessary for the progressive growth of a tumor, it should be possible to demonstrate the number of factors which are derived from the two original stocks, in this case the D and the A stocks. This procedure could not be followed for tumors 13714B and 13714A, as the number of individuals in the two back-cross generations was insufficient for the calculations.

Tumor 13714BX (or BX) was inoculated into 67 individuals of the 1BC generation; the observations were: $23 + : 44 - \pm 2.59$; the percentage negative was: $65.67\% \pm 3.87$.

Table IV, Ratios 4 to 6, compares the results in the 1BC generation for this tumor with the expectation for 3 and 4 factors being brought into the cross by the D stock. The comparison between the observation and expectation for 3 factors gives a difference of $9.33\% \pm 5.22$ or $1.79 \times \text{P.E.}$ and for 4 factors $15.67\% \pm 5.61$ or $2.79 \times \text{P.E.}$ Thus it is demonstrated that 3 or 4 factors are brought into the cross by the D stock.

The comparisons are made for the ZBC generation in Table IV, Ratios 7 to 9. The observation ($92.74\% \pm 0.81$), when compared with the expectation for one factor being carried by the A stock ($93.75\% \pm 0.75$), gives a difference of $1.01\% \pm 1.10$ or $0.92 \times \text{P.E.}$ Thus it is proved that if 5 dominant mendelian factors are involved for susceptibility to tumor 13714BX (or BX), then the A stock carries 1 and the D stock 3 and possibly 4 of the 5 factors.

For Tumor 13714AX (or AX): The results for the inoculation of tumor 13714AX in the F_2 generation were: $61 + : 344 - \pm 4.80$; the percentage negative was: $84.94\% \pm 1.19$ (Table I).

The observation, compared with the expectation for 5 factors ($76.27\% \pm 1.41$), shows a difference of $8.67\% \pm 1.55$ or $5.60 \times \text{P.E.}$; for 6 factors, a difference of $2.73\% \pm 1.74$ or $1.57 \times \text{P.E.}$; for 7 factors, a difference of $1.71\% \pm 1.64$ or $1.04 \times \text{P.E.}$; and for 8 factors ($89.99\% \pm 0.89$), a difference of $5.05\% \pm 1.48$ or $3.41 \times \text{P.E.}$ (Table V, Ratios 1 to 3).

The comparison between the observed data for the 1BC generation ($79.10\% \pm 3.31$) and the expectation for 4 factors being carried by the D stock ($87.50\% \pm 2.71$) shows a difference of $8.40\% \pm 4.28$ or $1.92 \times \text{P.E.}$; and for 5 factors ($75.00\% \pm 3.52$) a difference of $4.10\% \pm 4.83$ or $0.85 \times \text{P.E.}$ (Table V, Ratios 4 to 6).

TABLE V

Comparison of the Observed Data for the F_2 Generation with the Expectation for 6 and 7 Dominant Mendelian Factors for Tumor 13714AX (Ratios 1 to 3); for the 1BC Generation with the Expectation for 4 and 5 Factors Being Brought into the Cross by the D Stock (Ratios 4 to 6) and for the ZBC Generation with the Expectation for 1 and 2 Factors Being Brought into the Cross by the A Stock (Ratios 7 to 9)

Ra- tio	Tumor 13714AX	Gener- ation	Number of Individuals	Per Cent Negative	Difference Between Ratios
1	Observation	F_2	$61.00+ : 344.00- \pm 4.80$	$84.94\% \pm 1.19$	
2	Expectation 6 Factors	F_2	$72.07+ : 332.94- \pm 5.13$	$82.21\% \pm 1.27$	1 and 2 = $2.73\% \pm 1.74$ or $1.57 \times \text{P.E.}$
3	Expectation 7 Factors	F_2	$54.07+ : 350.93- \pm 4.56$	$86.85\% \pm 1.13$	1 and 3 = $1.71\% \pm 1.64$ or $1.04 \times \text{P.E.}$
4	Observation	1BC	$14.00+ : 53.00- \pm 2.20$	$79.10\% \pm 3.31$	
5	Expectation 4 Factors	1BC	$8.38+ : 58.62- \pm 1.81$	$87.50\% \pm 2.71$	4 and 5 = $8.40\% \pm 4.28$ or $1.92 \times \text{P.E.}$
6	Expectation 5 Factors	1BC	$16.75+ : 50.25- \pm 2.36$	$75.00\% \pm 3.52$	4 and 6 = $4.10\% \pm 4.83$ or $0.85 \times \text{P.E.}$
7	Observation	ZBC	$13.00+ : 507.00- \pm 2.37$	$97.50\% \pm 0.46$	
8	Expectation 1 Factor	ZBC	$8.13+ : 511.87- \pm 1.89$	$98.44\% \pm 0.37$	7 and 8 = $0.94\% \pm 0.59$ or $1.59 \times \text{P.E.}$
9	Expectation 2 Factors	ZBC	$16.26+ : 503.74- \pm 2.65$	$96.88\% \pm 0.52$	7 and 9 = $0.62\% \pm 0.72$ or $0.89 \times \text{P.E.}$

The ZBC data ($97.50\% \pm 0.46$) when compared with the expectation for 1 factor from the A stock ($98.44\% \pm 0.37$) gives a difference of $0.94\% \pm 0.59$ or $1.59 \times \text{P.E.}$, and for 2 factors ($96.88\% \pm 0.52$) a difference of $0.62\% \pm 0.72$ or $0.89 \times \text{P.E.}$

Therefore, if we assume that for susceptibility to tumor 13714AX (or AX) 7 dominant mendelian factors are necessary, then 4 or 5, probably 5, are carried by the D stock and one or 2 factors, probably 2, are carried by the A stock.

Comparison of the Various Tumors

As the two original tumors arose spontaneously and independently of each other in the same F_1 individual, a comparison should be made of the results secured from the inoculation of the various tumors in the F_2 generation. Every animal was inoculated simultaneously with two tumors. These comparisons are tabulated in Table VI.

TABLE VI

Comparisons for the Observations Secured from the Inoculation of the Various Tumors in the F_2 Generation

Ratio	Tumor	Number of Mice	Per Cent Negative	Difference Between Ratios
1	13714B	11+ : 262- ± 2.17	95.97% ± 0.79	1 and 2 = 0.22% ± 1.21 or 0.18 $\times$ P.E.
2	13714A	9+ : 203- ± 1.93	95.75% ± 0.92	
3	13714B	11+ : 262- ± 2.17	95.97% ± 0.79	3 and 4 = 23.30% ± 1.76 or 13.24 $\times$ P.E.
4	13714BX	94+ : 250- ± 5.51	72.67% ± 1.57	
5	13714A	9+ : 203- ± 1.93	95.75% ± 0.92	5 and 6 = 10.81% ± 1.50 or 7.21 $\times$ P.E.
6	13714AX	61+ : 344- ± 4.80	84.94% ± 1.19	
7	13714BX	94+ : 250- ± 5.51	72.67% ± 1.57	7 and 8 = 12.27% ± 1.99 or 6.17 $\times$ P.E.
8	13714AX	61+ : 344- ± 4.80	84.94% ± 1.19	

The results secured from the inoculation of tumor 13714B (95.97% ± 0.79) compared with those secured when tumor 13714A was used (95.75% ± 0.92) give a difference of 0.22% ± 1.21 or 0.18 $\times$ P.E.

Tumor 13714BX (72.67% ± 1.57), compared with tumor 13714B, gives a difference of 23.30% ± 1.76 or 13.24 $\times$ P.E., a very significant variation, although tumor 13714BX was obtained from tumor 13714B during the process of transplantation. Likewise, observations for the tumor 13714AX (84.94% ± 1.19) compared with those secured for tumor 13714A show a significant difference: 10.81% ± 1.50 or 7.21 $\times$ P.E. When a comparison is made between tumors 13714BX and 13714AX the difference is 12.27% ± 1.99 or 6.17 $\times$ P.E.

All the individuals of the F_2 generation were inoculated simultaneously with two tumors throughout the experiment. For the simultaneous inoculation of tumor 13714B and tumor 13714A we secured 5 + B : + A, 3 + B : - A and 4 - B : + A; of tumor 13714B and tumor 13714AX: 3 + B : + AX, and 5 - B : + AX; of tumor 13714BX and tumor 13714AX: 53 + BX : - AX, and 41 + BX : + AX.

Assuming the presence of 5 factors for tumor 13714BX and 7

for tumor 13714AX, there would be a difference of 2 factors in the F_2 generation or $9+ : 7$ (Table II). In the F_2 generation the observation was $53 + BX : + AX$ to $41 + BX : - AX \pm 3.31$; the percentage negative was $43.66\% \pm 3.47$. According to the expectation for a 2-factor difference, we would have: $52.81 + BX : + AX$ to $41.19 + BX : - AX \pm 3.15$; the percentage negative: $43.75\% \pm 3.28$. The difference between the observation and expectation is $0.09\% \pm 4.78$ or $0.02 \times \text{P.E.}$ (Table VII).

TABLE VII

Comparison between the F_2 Individuals Growing the Various Tumors as Indicated, Which Were Inoculated Simultaneously (A comparison is also given between the observed reaction with the expectation for the simultaneous inoculation of the tumors 13714BX and 13714AX, the difference in the number of factors involved probably being 2 or $9+ : 7-$)

Tumors inoculated : B and A		
$+B : +A$	$+B : -A$	$-B : +A$
5	3	4
Tumors inoculated : B and AX		
$+B : +AX$	$+B : -AX$	$-B : +AX$
3	0	5
Tumors inoculated : BX and AX		
$+BX : +AX$	$+BX : -AX$	% Negative
Observation	$53.00+ : 41.00- \pm 3.31$	$43.66\% \pm 3.47$
Expectation	$52.81+ : 41.19- \pm 3.15$	$43.75\% \pm 3.28$
$(9+ : 7-)$		
Difference = $0.09\% \pm 4.78$ or $0.02 \times \text{P.E.}$		

The curves representing the percentage of susceptible individuals resulting from the inoculation of the various tumors in the F_2 generation are compared in Fig. 10.

Summary

The two original tumors arose spontaneously in the same F_1 mouse, No. 13714. When inoculated into F_2 individuals, both tumors (13714A and 13714B) required the simultaneous presence of 11 dominant mendelian factors for continued growth. The factors were evidently not the same for both tumors, as some F_2 individuals were susceptible to the B tumor but not to the A tumor; others grew the A tumor but not the B tumor, while still others grew both tumors progressively. During the process of transplantation both tumors "broke down," giving smaller numbers of segregating mendelian units. The "mutant" of the B tumor, 13714BX, gave a 5-factor ratio, 3 and possibly 4 factors being

derived from the original D parent, while the other factor was derived from the A parent. Tumor 13714AX gave a 7-factor ratio, 4 or 5 factors, probably 5, being contributed by the D stock and 1 or 2, probably 2, by the A stock. All mice of the generation which grew tumor 13714AX were also susceptible to tumor 13714BX. However, some grew the BX tumor but not the AX. Therefore, 5 of the 7 factors necessary for susceptibility to tumor 13714AX are identical with the 5 factors required for the growth of tumor 13714BX.

These data are briefly summarized in Table VIII.

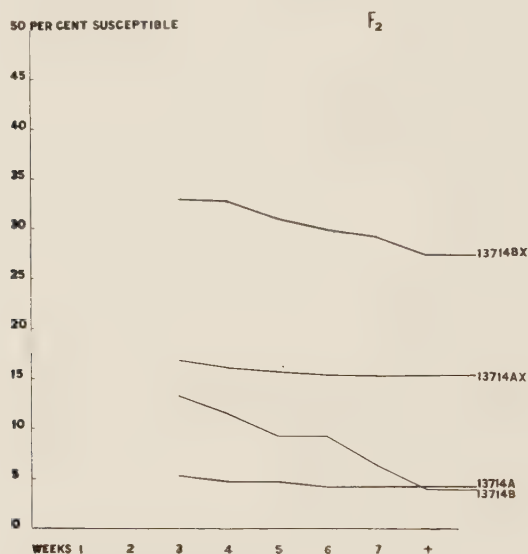


FIG. 10. CURVES REPRESENTING THE PERCENTAGE OF SUSCEPTIBLE INDIVIDUALS IN THE F₂ GENERATION RESULTING FROM THE INOCULATION OF THE VARIOUS TUMORS

DISCUSSION

The results obtained in the transplantation of neoplastic tissue have a direct bearing on many biological problems. The most obvious application of such data is without doubt to the study of the so-called cancer process. By this is meant those changes that are encountered within the organism during the time a cancer or other tumor formation is being brought about. A discussion of the results obtained in this experiment may make this point clearer.

The present investigation consisted of a comparative study of the transplantation of two tumors that arose spontaneously, yet independently of each other, in a single individual. This mouse,

No. 13714, belonged to the first filial generation made by mating originally mice of two distinctive inbred stocks.

The nature of the origin of spontaneous cancer is still an unsettled question. However, it is safe to assume, from the study of morphogenesis and genetics, that the cells of the body which give rise to tumors of the same fundamental tissue in the same individual are biologically identical and endowed with the same genetic constitution. Assuming this to be the case, one would expect that two tumors of the same histological appearance, arising in the same organism at approximately the same time,

TABLE VIII

Probable Number of Factors for the Various Tumors and the Number Derived from the Parent Stocks Where They Could be Determined

Tumor	Factors in F ₂ Generation	Factors from D Parent	Factors from A Parent
13714B	11 (?)	?	?
13714BX	5	3 or 4	1
13714A	11 (?)	?	?
13714AX	7	5	2

would give identical results when inoculated simultaneously into the same series of hosts. This result is seldom if ever realized. Tumors from the same host are endowed with a great degree of physiological individuality (Strong, 1929).

The F₁ generation made by crossing two highly inbred stocks of mice would be heterozygous for a large number of genetic factors. Homozygous genes might exist if the two original parents carried the same factors. Regardless of the composition of the parent stocks, all the F₁ individuals would be genetically the same, provided each of the parent stocks were inbred enough to be homozygous.

The two original tumors gave practically the same results when inoculated into representatives of the various pure stocks and hybrid generations (Table I). With one exception, all the individuals of the first filial generation were susceptible both to tumor 13714B and tumor 13714A. Individuals of the two parental stocks were consistently negative to the grafts. The susceptible individuals of the Murray dilute brown strain cannot be considered as significant, as previous work indicates that the two derivatives of the dilute brown stock are probably not genetically the same.

The percentage of susceptible individuals in the F₂ generation

was the same for both tumors. Likewise, the number of dominant mendelian factors necessary for progressive growth was the same for both tumor 13714B and tumor 13714A (Table III).

These results are comparable to those secured by Tyzzer, Little, and Strong in their various experiments on pure stocks. They differ in that in all of the above problems the tumors employed arose in an individual of one of the pure parental stocks of mice.

Although the number of individuals in the two back-cross generations (1BC and ZBC) is small, we were able to determine that one of the parent stocks did not contribute all the factors necessary for the progressive growth of either tumor. From genetic principles we are able to demonstrate that if all the susceptibility factors had been contributed from either parental stock, all the animals of the significant back-cross generation would have been susceptible (Little and Tyzzer, 1916).

The two tumors arose spontaneously and independently of each other in the same individual; they gave the same factor ratio when inoculated into individuals of the same F_2 generation; and yet they had different physiological reactions. Some F_2 individuals were susceptible to both tumor 13714B and tumor 13714A; others were susceptible to tumor 13714B but resistant to tumor 13714A, and still others negative to tumor 13714B but susceptible to tumor 13714A (Table VII). This finding is unquestionably significant, since all negative mice were reinoculated with tissue from the same tumor.

The genetic constitution of all the cells of an individual probably remains the same. All the cells of the body are believed to have been originally biologically the same, being differentiated by various processes from the fertilized egg. Morphogenesis takes place not so much by the spontaneous deviations within the genetic constitution of the individual cells as by other processes, indicated by cell relationship, which is not much understood at present. This statement, based upon the careful observations of many cytologists, must now be somewhat modified. Wright and Eaton (1926) have described the occurrence of a "somatic mutation" during the early development of a guinea-pig which affected localized areas of the adult coat pattern. Similar results have been described in other forms by several investigators. That differentiation may take place by "somatic mutation" has been recently maintained by Davenport in his presidential address

before the Society of Zoologists at Des Moines. These later developments of morphogenesis do not invalidate the arguments for the specific genetic constitution of tissue cells emphasized in this paper.

The author has determined (1930) that if multiple grafts of the same healthy neoplastic tissue are transplanted, the host reacts by either growing all or none. Thus, if two different tumors are inoculated simultaneously into the same host, differences in susceptibility to the tumors must be inherent within the tumor cell, the host remaining the same. Since the tumors employed in the present problem were inoculated simultaneously in the same animals, the factors necessary for the progressive growth of each tumor could not have been identical, although the two tumors, 13714B and 13734A, gave the same factor ratio. Had they been the same, all mice susceptible to one tumor would likewise have grown the other.

If we say that the genetic constitution of one tumor was the same as the host which gave rise to it, then the genetic constitution of the other tumor has deviated from that condition sometime during the process of the formation of cancerous tissue.

During the transplantation of the original tumors it was observed that the percentage of susceptible individuals in the F_2 generation showed a decided increase after a few transfer generations. Previous to this change in susceptibility the results had been uniform. The new percentages were maintained during the remainder of the experiment. Not only was there a change in the reaction potential existing between the tumors and hosts, but there was also an increased proliferative vigor of the tumors.

Tumor 13714BX (formerly called 13714B) gave now a five factor ratio in the F_2 generation (Table IV) and the tumor 13714AX (formerly called 13714A) a seven factor ratio (Table V). As the number of individuals of the backcross generations was large, we were able to determine that of the five factors necessary for the progressive growth of tumor 13714BX, only one was contributed by the A parent and three or four by the D parent (Table IV). The D stock contributed five and the A stock two of the seven factors necessary for susceptibility to the tumor 13714AX (Table V).

Comparison of the results secured from the inoculation of some of the various tumors (B and BX, A and AX, and BX and AX, Table VI) in the F_2 generation gave data which were mathe-

matically significant. Many of the F_2 mice were susceptible to both tumors BX and AX and some to tumor BX but not tumor AX. In no instance were mice observed which were negative to tumor 13714BX but susceptible to tumor 13714AX. This showed that the five factors necessary for the progressive growth of tumor 13714BX were identical with five of the seven factors necessary for susceptibility to tumor 13714AX. The comparison of the observation and expectation for a two-factor ratio between the BX tumor and the AX tumor gave results which were not, however, significant (Table VII).

Three theories have been formulated to explain results such as we have obtained: (1) the virulence theory (Ehrlich and Apolant), (2) the adaptation theory (Bashford, Murray, Haaland, Bowen, Cramer and Woglom), and (3) the "mutation theory" (Strong).

According to the first two theories any variation encountered in the percentage of susceptible individuals in the various stocks and generations would be gradual and cumulative. Since these theories have no genetic foundation, the changes should also have been observed in the original parental stocks as well as in the F_2 generation. Both tumors gave sudden changes in their reaction potential which remained constant. Thus it is evident that the data at hand can not be explained by either the virulence or adaptation theory.

Before considering the third theory ("mutation") it may be well to recall the fundamental facts of transplantation. The cell is the unit of structure of the tumor mass, as well as of the entire normal organism. The inoculation of neoplastic tissue is the transplantation of cells, not an infectious process. Of the bit of tissue which is transplanted, only a few cells remain alive to give rise to the resulting growth. With these well established points before us, we are able to discuss this change of transplantability encountered in cancerous tissue.

The results were due either to changes within the hosts' cells or deviations within the tumor cells. It is obvious that the genetic constitution of the hosts did not alter sufficiently during the investigation to cause the variations observed. Since the two tumors were compared in the same series of animals, any potential change within the host could not explain the results. The genetic constitution of the tissue around the left and the right axilla of a mouse is unquestionably the same.

The other alternative is that the tumor cell has changed. This might have occurred by any of several methods. (1) The original mass may have been a mixed tumor, the cells of which had various proliferative energy capacities and different degrees of tissue specificity. This is improbable, since in order to produce the observed results the cells of the different types would have had to remain together during several inoculations. Many other objections to this interpretation have already been discussed by Strong (1926 a and b). (2) The changes may have resulted from the inoculation of a new tumor arising in the host from which the tissue for transplantation was obtained. However, the tumors were histologically indistinguishable, and the donors were usually males, which have very few spontaneous tumors.

(3) The only other theory which will explain the observation is that changes have occurred within the genetic constitution of the tumor cell, changes which were inherited and perpetuated by cell division. In other words, "mutations" have occurred within the tumor cell during the process of transplantation, as maintained by Strong in various experiments (1926 a and b). Not only has there been a decrease in the degree of tissue specificity of the tumor cell, but an increase in the proliferative vigor as well.

From the above it is apparent that studies on the transplantation of cancerous tissue under controlled conditions do throw some light on the constitution of cancerous tissue and supply a theory for the interpretation of "probable" somatic changes in the incidence of cancer. Extreme caution must be employed, however, in the application of the phenomena associated with the transplantation of cancerous tissue to the problem of spontaneous neoplasms; the term "mutation" is not used in any causative sense. It may not even be involved in the origin of the cancerous tissue in the first place.

The problem of the transplantation of neoplastic tissue may add difficulties to the cancer problem, as maintained by several pathologists. Nevertheless, in every experiment where pedigreed animals have been used, the data obtained have added support to the genetic theory of transplantation as advanced by Little and Strong (1924). Also, by the inoculation of cancerous tissue we have a method whereby it is possible to study, by genetic methods, certain of the physiological characteristics as possessed by the individual. This study indicates that the reaction of the host to an implanted piece of neoplastic tissue is controlled by the nature

of its genetic constitution determined by the zygote which gave rise to it.

The organism is the final result of the zygote formed by the combination of two gametes. As the result of a differentiation of the cells, resulting from the division of the zygote, there are produced the various tissues and organs which make up the body. These have been grouped as reproductive tissue and soma. The prevailing opinion maintains that, since the soma is the result of differentiation from identical cells, the organism may be considered as a whole. The cells, according to this view, must be biologically and genetically the same throughout the individual.

Assuming this to be true, one might expect that tissues, such as cancer, arising from one or more of these cells, would possess the same physiological characteristics and genetic constitution as the original cell or cells from which they are derived. That such is not the case has been mentioned previously. If we maintain that the organism is a unit, we must qualify this statement by adding that the unit is made up of cells, possibly biologically and genetically the same, but endowed with the power of differentiating into other cells (as cancer) which deviate from the original cellular constitution of definitive or embryonic cells.

Since the evidence in the present investigation shows that neoplastic tissues arising in the same individual possess different physiological and genetic characteristics, at least during the process of transplantation, is it not conceivable that spontaneous tumors may likewise result by a method comparable to "somatic mutation"?

As Strong has previously pointed out: "The problem of the transplantation of cancer belongs to the domain of genetics and may eventually be solved by genetic methods" (1926c).

CONCLUSIONS

1. The transplantation of spontaneous tumors arising in hybrid mice supports the genetic theory of transplantation.
2. Tumors arising spontaneously yet independently of each other in a single individual may call forth the same factorial complex on the part of the host but at the same time show different physiological characteristics. This phenomenon is encountered when the two tumors are inoculated simultaneously in the same series of mice.
3. Hereditary genetic changes, characteristic of somatic muta-

tions, may occur within the tumor cell during the process of transplantation.

4. The "mutant" tumors possess different physiological characteristics.

5. The hereditary genetic changes are perpetuated by cell division from one cell generation to the next.

6. The nature of the genetic changes is as yet undetermined.

7. The tumor cell may deviate from the genetic constitution of the individual which gave rise to it, at least during the process of transplantation.

8. The theory of mutation accounts for changes formerly explained by the theories of adaptation and virulence.

9. The difference between susceptibility and non-susceptibility to two of the transplanted tumors is due to the activity of two dominant mendelian factors.

10. The factors necessary for susceptibility to hybrid tumors may be derived from both of the original parent stocks.

11. The physiological characteristics of the F_1 hybrids are determined from characteristics derived from both parents.

12. The physiological characteristics of both the host and the tumor cell are controlled by intrinsic genetic factors.

LITERATURE CITED

- APOLANT, H.: *Ztschr. f. allg. Physiol.* 9: 535, 1909.
- BASHFORD, E. F.: Fourth Scientific Rep., Imperial Cancer Research Fund, London, 1911, p. 137.
- BASHFORD, E. F., AND MURRAY, J. A.: First Scientific Rep., Imperial Cancer Research Fund, London, 1904, p. 1.
- BASHFORD, E. F., MURRAY, J. A., AND BOWEN, W. H.: *Ztschr. f. Krebsforsch.* 5: 417, 1907.
- BASHFORD, E. F., MURRAY, J. A., AND BOWEN, W. H.: Third Scientific Rep., Imperial Cancer Research Fund, London, 1908, p. 284.
- BASHFORD, E. F., MURRAY, J. A., AND CRAMER, W.: Second Scientific Rep., Imperial Cancer Research Fund, London, 1905, p. 1.
- BASHFORD, E. F., MURRAY, J. A., AND CRAMER, W.: *Proc. Roy. Soc. ser. B*, 79: 164, 1907.
- BASHFORD, E. F., MURRAY, J. A., HAALAND, M., AND BOWEN, W. H.: Third Scientific Rep., Imperial Cancer Research Fund, London, 1908, p. 262.
- BASHFORD, E. F., AND RUSSELL, B. R. G.: *Proc. Roy. Soc., ser. B*, 82: 209, 1910.
- BITTNER, J. J.: *Mich. Acad. Sci. Pub.* 11: 349, 1929.
- BITTNER, J. J.: *J. Cancer Research* 14: 466, 1930.
- BORREL, A.: *Ann. de l'Inst. Pasteur* 17: 81, 1903.

- BORREL, A.: Bull. de l'Inst. Pasteur 5: 497, 1907.
BIDRÉ, J.: Ann. de l'Inst. Pasteur. 21: 760, 1907.
CUÉNOT, L., AND MERCIER, L.: Compt. rend. Soc. de biol. 67: 736, 1909.
CUÉNOT, L., AND MERCIER, L.: Compt. rend. Acad. de sci. 150: 1443, 1910; 154: 784, 1912.
EHRlich, P.: Arb. a. d. Königl. Inst. f. Exp. Therap. 1: 75, 1906. Cited by Strong.
EHRlich, P.: Ztschr. f. Krebsforsch. 5: 59, 1907.
EHRlich, P.: Verhandl. d. deutsche path. Gesellsch. 13: 13, 1908. Cited by Woglom.
EHRlich, P., AND APOLANT, H.: Berl. klin. Wehnschr. 42: 871, 1905.
v. EISELBERG, F.: Wien. klin. Wehnschr. 3: 927, 1890. Cited by Woglom.
FICHERA: J. Exper. Med. 14: 320, 1911. Cited by Woglom from Apolant.
FIRKET: Bull. d. Acad. d. méd. 6: 1147, 1892.
FLEISHER, M. S., AND LOEB, L.: J. Cancer Research 1: 331, 1916.
GAY, F. P.: Proc. Soc. Exper. Biol. & Med. 6: 74, 1908-09.
GUSSIO, S.: Tumori 1: 75, 1911.
HAALAND, M.: Berl. klin. Wehnschr. 44: 713, 1907.
HANAU: Arch. f. klin. Chir. 39: 678, 1889. Cited by Woglom.
HERTWIG, O., AND POLL, H.: Abhandl. d. k. preuss. Akad. d. Wissensch. 1: 1, 1907. Cited by Woglom.
HERZOG, M.: J. Med. Research 3: 74, 1902.
JENSEN, C. O.: Centralbl. f. Bakteriöl. 34: 122, 1903.
JONES, E.: J. Cancer Research 10: 435, 1926.
KROSS, I.: J. Cancer Research 6: 25, 1921.
LAMBERT, R. A., AND HANES, F. M.: Proc. Soc. Exper. Biol. & Med. 8: 123, 1911.
LITTLE, C. C.: Science 40: 904, 1914.
LITTLE, C. C.: J. Exper. Zool. 31: 307, 1920.
LITTLE, C. C.: J. Cancer Research 8: 75, 1924.
LITTLE, C. C., AND STRONG, L. C.: J. Exper. Zool. 41: 93, 1924.
LITTLE, C. C., AND TYZZER, E. E.: J. Med. Research 33: 393, 1916.
LOEB, L.: J. Med. Research 1: 28, 1901; 3: 44, 1902; 34: 39, 1918.
LOEB, L.: Am. Med. 10: 265, 1905.
LOEB, L.: Am. Nat. 54: 45, 1920.
LOEB, L.: Biol. Bull. 40: 147, 1921.
LOEB, L., AND FLEISCHER, N. S.: Centralbl. f. Bakteriöl. 67: 135, 1912.
LOEB, L., AND WRIGHT, S.: Am. J. Path. 3: 251, 1927.
MORAU, H.: Compt. rend. Soc. de biol. 3: 721, 1891.
MORAU, H.: Compt. rend. Acad. d. sc. 117: 62, 1893.
MORAU, H.: Arch. d. méd. expér. et d'anat. path. 6: 677, 1894.
NOVINSKY, M.: Centralbl. f. med. Wissensch. 14: 790, 1876. Cited by Woglom.
PFEIFFER, L.: Centralbl. f. Bakteriöl. 8: 794, 1890. Cited by Woglom.
PITZMAN, M.: Ztschr. f. Krebsforsch. 14: 57, 1914.
PRIME, F.: J. Cancer Research 5: 82, 1920.
ROUS, P., AND LANGE, L. B.: J. Exper. Med. 20: 413, 1914.

- RUSSELL, B. R. G.: Fifth Scientific Rept., Imperial Cancer Research Fund, London, 1912, p. 1.
- SITTENFIELD, M. J.: Proc. Soc. Exper. Biol. & Med. 9: 56, 1911-12.
- STAHR, H.: Centralbl. f. allg. Path. u. path. Anat. 20: 628, 1909. Cited by Woglom.
- STRONG, L. C.: J. Exper. Zool. 36: 67, 1922.
- STRONG, L. C.: J. Exper. Med. 39: 447, 1924; 43: 713, 1926.
- STRONG, L. C.: Genetics 11: 294, 1926.
- STRONG, L. C.: Am. Nat. 60: 201, 1926.
- STRONG, L. C.: J. Exper. Zool. 45: 231, 1926.
- STRONG, L. C.: J. Cancer Research 10: 219, 1926; 13: 103, 1929.
- SUGIURA, K.: J. Cancer Research 12: 143, 1928.
- SUGIURA, K., AND BENEDICT, S. R.: J. Cancer Research 5: 373, 1920; 13: 340, 1929.
- SUGIURA, K., NOYES, H. M., AND FALK, K. G.: J. Cancer Research 6: 285, 1921.
- TYZZER, E. E.: J. Med. Research 16: 519, 1909.
- VAN ALSTYNE, E. V., AND BEEBE, S. P.: J. Med. Research 24: 217, 1913-14.
- VELICH, A.: Wien. med. Blätter. 21: 711, 1898. Cited by Woglom.
- WOGLOM, W. H.: The Study of Experimental Cancer, Columbia University Press, 1913.
- WOGLOM, W. H.: J. Exper. Med. 22: 766, 1915.
- WOGLOM, W. H.: J. Cancer Research 4: 1, 1919.
- WOGLOM, W. H.: Cancer Rev. 4: 129, 1929.
- WOOD, F. C., AND CURTIS, M. R.: Proc. N. Y. Path. Soc. n.s. 22: 179, 1922.
- WRIGHT, S., AND EATON, O. N.: Genetics 11: 333, 1926.

